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Steroidal Sapogenins. XI.² Steroidal C-Ring Lactones

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Contrary to reports by other workers we have found that acid-catalyzed perbenzoic acid and peracetic acid oxidation of steroidal C₁₂-ketosapogenins results in oxidative attack on the C-ring with ϵ -lactone formation. The introduction of oxygen led to only one of the two possible isomeric lactones, *vis.*, the lactone of the 12,13-secospirostane series. The sapogenin side chain was unaffected by the reagent. The bile acid, methyl 3 α -carbethoxyoxy-12-ketocholanoate, gave similar results, but C₁₁-ketosteroids resisted attack.

Oxidation of steroid carbonyl groups at C₃, C₇, C₁₇ and C₂₀ by peracids has been reported by various workers.³⁻⁷ On the other hand, the less

reactive carbonyl groups at C₁₁ and C₁₂ are described as inert⁸ to this type of reagent. We have found conditions for reaction not only of the C₁₂ keto group of methyl 3 α -carbethoxyoxy-12-ketocholanoate, but also for reaction of the more hindered C₁₂ keto group of the sapogenin, hecogenin acetate (5 α ,22 α -spirostan-3 β -ol-12-one 3-acetate). Under non-anhydrous reaction conditions using perbenzoic acid or peracetic acid in the presence of a catalytic amount of sulfuric acid and a reaction period of several days, seven-membered C-ring lactones were obtained in high yield. In this manner the oxidation of hecogenin acetate led to the formation of a single lactonic product

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(2) Paper X, E. S. Rothman, M. E. Wall and C. R. Eddy, *THIS JOURNAL*, **75**, 6325 (1953).

(3) L. Ruzicka, V. Prelog, *et al.*, *Helv. Chim. Acta*, **28**, 618, 1651 (1945).

(4) H. Heymann and L. F. Fieser, *ibid.*, **35**, 631 (1952).

(5) R. P. Jacobsen, *et al.*, *J. Biol. Chem.*, **171**, 61, 71, 81 (1947).

(6) H. Heusser, A. Segre and P. A. Plattner, *Helv. Chim. Acta*, **31**, 1183 (1948).

(7) T. F. Gallagher and T. H. Kritchevsky, *THIS JOURNAL*, **72**, 882 (1950); R. B. Turner, *ibid.*, **72**, 878 (1950).

(8) V. Burckhardt and T. Reichstein, *Helv. Chim. Acta*, **25**, 821, 1434 (1942); *cf.* L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, pp. 237, 402.

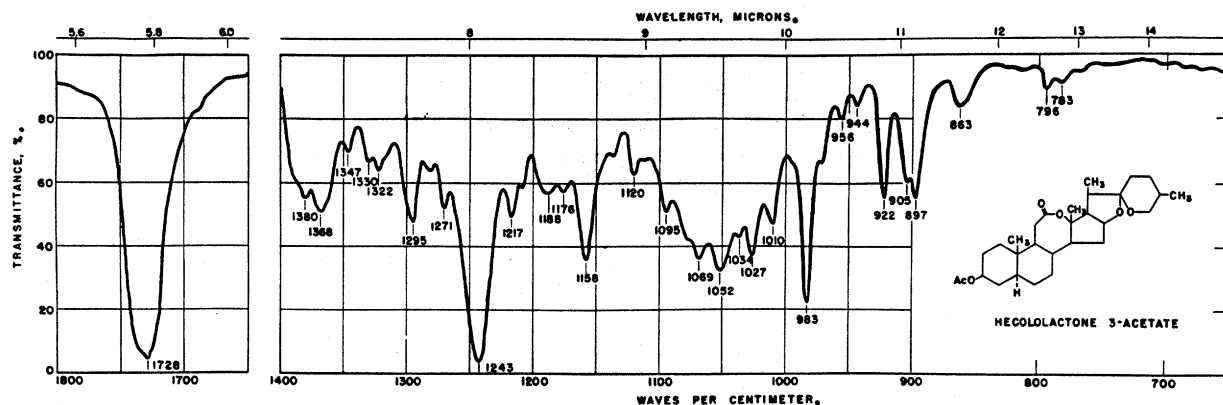
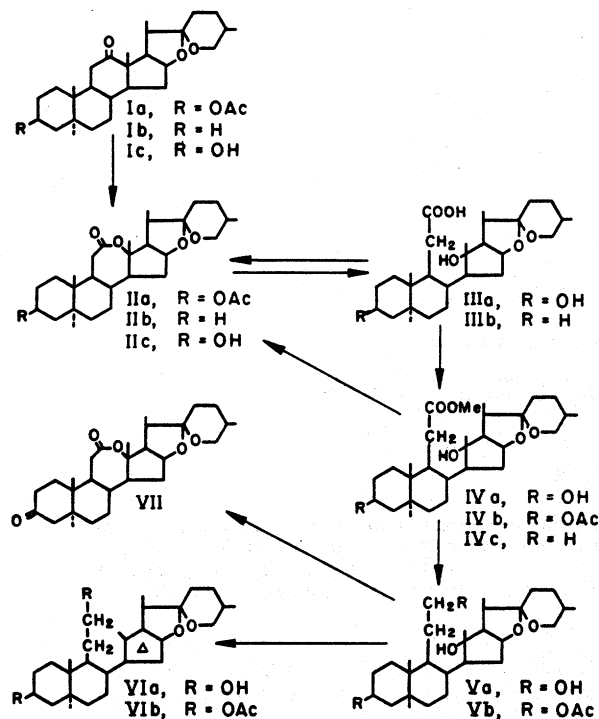


Fig. 1.—Infrared absorption spectrum of hecololactone 3-acetate (IIa) (CS_2 solution, 6.7 g./l.; 1.0 mm. cell).

unaltered in the spiroketal side chain. We formulate this product as IIa and assign it the trivial name of hecololactone 3-acetate. The systematic name⁹ is $3\beta,13\text{-}\xi$ -dihydroxy-12,13-seco- $5\alpha,22\alpha$ -spirostan-12-oic-acid 12,13-lactone 3-acetate. Under the same conditions of reaction C_{11} -ketosteroids failed to react in 30 days.



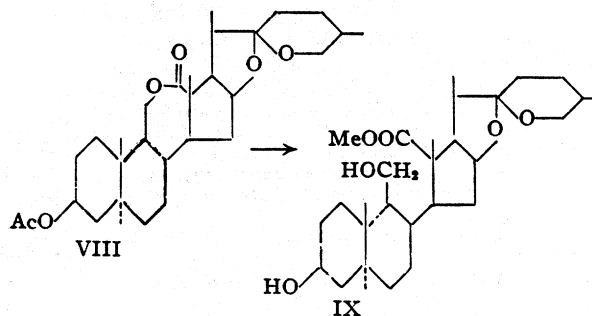
Hecololactone 3-acetate was best prepared by reaction of hecogenin acetate, Ia, with perbenzoic acid in chloroform solution containing 0.3 volume percentage of sulfuric acid. The reaction proceeded smoothly at room temperature giving 80% yields in 60 hours and 98% yields in 12 days. Hecololactone 3-acetate also could be prepared in lower yields using a mixture of 30% aqueous commercial hydrogen peroxide in acetic acid medium. Use of pre-formed commercial peracetic acid in acetic acid gave poor yields. Jacobsen⁵ reported

(9) Nomenclature recommended by International Union of Pure and Applied Chemistry, Division of Chemistry and Chemical Technology, National Research Council.

similar difficulties with this reagent in oxidizing C_{17} -ketones.

Proof of Structure of Hecololactone 3-Acetate

The sharp melting points, failure to obtain two sets of derivatives, and behavior on chromatography indicate that the oxidation product IIa is a single compound rather than a mixture. Saponification of IIa gave hecolic acid, IIIa ($3\beta,13\text{-}\xi$ -dihydroxy-12,13-seco- $5\alpha,22\alpha$ -spirostan-12-oic acid). All attempts to acetylate hecolic acid resulted in ring closure and formation of hecololactone 3-acetate. When hecolic acid was heated *in vacuo* hecololactone, IIc, ($3\beta,13\text{-}\xi$ -dihydroxy-12,13-seco- $5\alpha,22\alpha$ -spirostan-12-oic acid 12,13-lactone) was formed. Esterification of hecolic acid with diazomethane gave methyl hecolate (IVa) (methyl $3\beta,13\text{-}\xi$ -dihydroxy-12,13-seco- $5\alpha,22\alpha$ -spirostan-12-carboxylate). Acetylation by the analytical method of Ogg, Porter and Willits¹⁰ showed that only one of the two hydroxyl groups present was acetylable. The product, methyl hecolate 3-acetate (IVb) (methyl $3\beta,13\text{-}\xi$ -dihydroxy-12,13-seco- $5\alpha,22\alpha$ -spirostan-12-carboxylate 3-acetate) showed infrared absorption bands in the 3500 cm^{-1} region characteristic of hydroxyl groups and a band at 1245 cm^{-1} of strength corresponding to only one acetate group per molecule. An alternative formulation of hecololactone 3-acetate as VIII must be rejected since saponification and treatment with diazomethane to form IX leaves no explanation for (1) failure to acetylate completely, (2) ease of dehydration (see below).



Lithium aluminum hydride reduced methyl hecolate in high yield to triol Va, hecolyl alcohol,

(10) C. L. Ogg, W. L. Porter and C. O. Willits, *Anal. Ed., Ind. and Eng. Chem.*, **17**, 394 (1945).

(12,13-seco-5 α -22a-spirostan-3 β ,12,13 ξ -triol) which had one non-acetyltable hydroxyl group. This compound formed, on acetylation the hydroxy diacetate Vb (12,13-seco-5 α ,22a-spirostan-3 β ,12,13 ξ -triol 3,12-diacetate). Final proof of the tertiary nature of the C-13 hydroxyl group of hecolyl alcohol was its facile dehydration by traces of mineral acid or by heat to a dihydroxy olefin, VIa, anhydro-hecolyl alcohol (12,13-seco-5 α -22a-spirost- ξ -ene-3 β ,12-diol).

Figure 2 shows a portion of the infrared spectra of Va (dotted line) with VIa (solid line). In the conversion of Va to VIa there is a marked loss of hydrogen-bonded hydroxyl groups (absorbing near 3400 cm^{-1}), with only a small change in non-bonded hydroxyl groups (absorbing near 3600 cm^{-1}). This suggests that the C₁₃-hydroxyl of VIa is hindered from intermolecular hydrogen bonding and that in Va, this hydroxyl forms primarily intramolecular hydrogen bonds with the C₁₂-hydroxyl. The diacetate VIb was free of hydroxyl groups.

The double bond in VI has not been definitely located, but the following evidence indicates its presence in the D-ring. The olefin gave a negative Tortelli-Jaffe test and a yellow color with tetranitromethane. The olefinic bond resisted catalytic hydrogenation (Adams catalyst in acetic acid, atmospheric pressure) and resisted oxidation by potassium permanganate both in pyridine and in aqueous potassium hydroxide-dioxane media. Addition of one mole of bromine occurred, however, under non-substitution conditions,¹¹ and about one atom of oxygen was added by epoxidation with perbenzoic acid to form a product giving no color reaction with tetranitromethane. Unless inversion or racemization at C₁₃ occurred in the oxidation step, the rule of *trans* (ionic) elimination, if valid in this system, would locate the bond between C₁₃ and C₁₈; however, the lack of reactivity of the double bond is behavior typical of hindered ethylenic linkages.¹² This fact and the lack of terminal methylene vibrations in the infrared spectrum require the placing of the double bond at C₁₃-C₁₄ or C₁₃-C₁₇ in the D-ring. The authors in reference 7 have shown that perbenzoic acid oxidation of the 1-acetyl-2-methylcyclohexanes and 20-ketosteroids proceeds without configurational change.

The ease of lactonization of hecolic acid involving the internal esterification of the carboxyl group with a tertiary non-acetyltable hydroxyl group may appear unusual; however, Jacobsen⁶ and Westerfeld¹³ report such behavior for the methyl ester of estrolic acid wherein a C₁₃-tertiary hydroxy-C₁₇ carbomethoxy compound lactonized in several attempts to remove the tertiary hydroxyl group by dehydration. We obtained lactone formation on attempts to acetylate both hecolic acid and its methyl ester. Oxidation of hecolyl alcohol under

(11) The brominated product contained no bromine in the side chain since it showed the usual spiroketal infrared absorption bands.¹⁷⁻¹⁸ 23-Bromohecolenin and 23,23-dibromosarasapogenin showed marked perturbation of these bands (unpublished observations, this Laboratory.)

(12) See *s.g.*, R. B. Turner in "Natural Products Related to Phenanthrene," L. F. Fieser and M. Fieser, Reinhold Publ. Corp., New York, N. Y., 1949, p. 624.

(13) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).

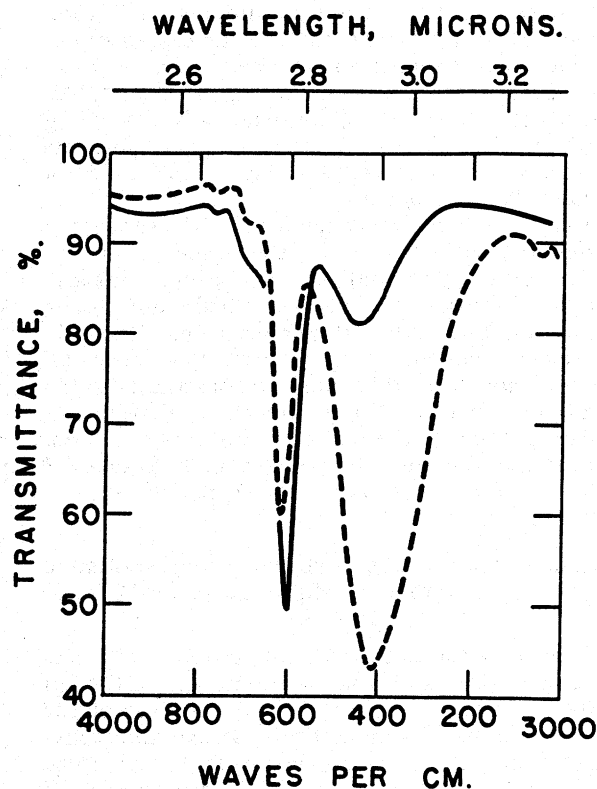


Fig. 2.—Infrared absorption spectra of: (1) anhydro-hecolyl alcohol (VIa), (solid line) chloroform solution, 19.2 g./kg. (ca. 28 g./l.), 0.5 mm. cell; (2) hecolyl alcohol (dotted line) chloroform solution, 25 g./l., 0.5 mm. cell.

mild conditions using chromium trioxide in pyridine^{14,15} also led to lactone formation.¹⁶

Evidence for unchanged E and F rings in hecololactone and its derivatives is found in the infrared spectra. Open C-ring derivatives showed the usual spiroketal bands^{17,18} with the 900 cm^{-1} band much stronger than the 920 cm^{-1} band, indicating an "iso" configuration. Lactonized C-ring derivatives showed these two bands nearly equal in strength, with the 900 band split into two components. See Fig. 1 as an example. This is not due to partial isomerization, since opening of the lactone ring by saponification gave a product with the usual "iso" type spectral bands, while the bands of the re-lactonized product were identical with those of the original lactone.

Rough estimates of the areas of these bands showed that, although the areas of the 900 and 920 bands of IIa were less and greater, respectively, than the corresponding bands of hecogenin acetate,¹⁸ the sum of the areas of the two bands was roughly the same for both compounds. This suggests that the altered 900 and 920 bands of the

(14) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *This Journal*, **75**, 422 (1953); H. H. Sisler, J. D. Bush and O. E. Accoun-tius, *ibid.*, **70**, 3827 (1948).

(15) Under these conditions we found that the 11 α -hydroxy group of 11 α -hydroxyprogesterone was nearly quantitatively converted to 11-keto-progesterone.

(16) The reaction possibly proceeded via the intermediate steps of aldehyde and cyclic hemiacetal formation.

(17) M. E. Wall, C. R. Eddy, M. L. McClennan and M. A. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

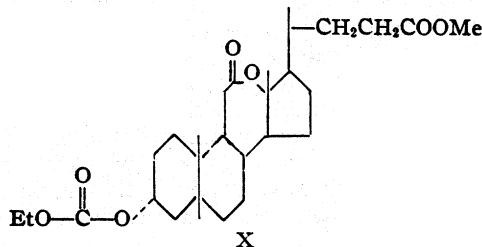
(18) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953).

C-lactones are due to the same intrinsic vibration as in ordinary sapogenins, rather than due to superposition of new bands from other centers in the molecule. Solvent environment also affects the relative intensities of these bands, non-polar solvents giving curves similar to Fig. 1 whereas polar solvents give curves midway between Fig. 1 and those of ordinary "iso" sapogenins.

From these facts, we conclude that the sapogenin lactone derivatives contain the same E and F ring structure as the natural "iso" sapogenins and that the alteration of the 900 and 920 bands is due to internal and external perturbation. Possibly the seven-membered ring distorts the spiroketal skeleton enough to add some new constraints to the vibrational modes of the spiroketal system. A similar splitting of the 900 band into two components is also observed with pennogenin 3-acetate,^{19,20} in which the EF ring vibrations are perturbed by a hydroxyl at C₁₇.

Characterization of the Perbenzoic Acid Oxidation Product of Methyl 3 α -Carbethoxyoxy-12-ketocholanate

We have found that the 12-keto group of a typical bile acid is also oxidizable to a lactone. Although we have not fully characterized derivatives of the lactone obtained from methyl 3 α -carbethoxyoxy-12-ketocholanate we have evidence that the lactone has the same 12,13-secosteroid structure described above for the hecogenin derivatives. Thus saponification to an acid, esterification with diazomethane, and lithium aluminum hydride reduction of the resulting ester gave a polyhydroxy product which upon acetylation gave a compound whose infrared spectrum showed the presence of one unacetylated, *i.e.*, probably tertiary, hydroxyl group. On this basis the structure X, methyl 3 α -carbethoxyoxy-13 ξ -hydroxy-12-carboxy-12,13-secocholanate 12,13-lactone, is tentatively assigned to the oxidation product of the bile acid.



We plan further experiments involving the degradation of the sapogenin C-ring lactones to analogs of progesterone and cortisone. Such C-ring oxygenated products might show interesting physiological activity.

Experimental

Hecolactone 3-Acetate, IIa (3 β ,13 ξ -Dihydroxy-12,13-seco-5 α ,22a-spirostan-12-oic-acid 12,13-Lactone 3-Acetate).—Hecogenin acetate (Ia), $[\alpha]^{25}_D -0.75$,²¹ m.p. 236–240°,

(19) Unpublished observations.

(20) "Collected Infrared Absorption Spectra of Steroid Sapogenins," by R. N. Jones, E. Katzenellenbogen and K. Dobriner, Division of Information Services, National Research Council, Ottawa, Canada, and Sloan-Kettering Institute for Cancer Research, New York, N. Y.

(21) All rotations were determined in a 1-dm. tube in chloroform solution 20 mg./3 ml. unless otherwise noted. All melting points were

5 g. was placed in a 125-ml. erlenmeyer flask surrounded with a melting-ice bath, and was covered with 58 ml. of a chloroform solution of perbenzoic acid (52 mg./ml.) prepared as described in reference 22 and often turbid with emulsified water. Two ml. of 10% sulfuric acid in acetic acid was added, the temperature allowed to rise to 25°, and the flask kept in the dark for the desired period. The progress of the oxidation was followed by removal of aliquots, which were washed with aq. sodium hydroxide and water, evaporated to dryness, and examined in the infrared for disappearance of the ketonic band at 1712 cm.⁻¹ and increase in the absorption near 1730 cm.⁻¹. The exact position of the latter band shifts with relative amounts of 3-acetate and lactone. The main reaction mixture was washed with water and dilute sodium hydroxide containing a little methanol to break emulsions. Evaporation gave a white crystalline residue of low solubility in methanol so that the product could be obtained in high purity from a single crystallization from that solvent. Purification of large scale preparations was effected by solution in hot chloroform, dilution with methanol and distillation of the chloroform. Alternatively purification could be effected by saponification to the water-soluble potassium salt of hecolic acid, *vide infra*, washing the aqueous alkaline solution with benzene to free of unreacted hecogenin, precipitation of hecolic acid with hydrochloric acid and acetylation to the lactone 3-acetate. The yield was 80% when the time allotted for the oxidation was 60 hours, and was 98% in 12 days. The product, rhombic tablets from methanol or acetone, melted at 292.0–292.5° after transition to regular hexagonal plates, $[\alpha]^{25}_D -65.1^\circ$.

Anal. Calcd. for C₂₈H₄₄O₆: C, 71.21; H, 9.21. Found: C, 71.28; H, 9.08.

Its infrared spectrum is shown in Fig. 1. The ratio of the area of the split 900 cm.⁻¹ band to that of the 920 cm.⁻¹ band is roughly 1.3 (on a scale of absorptivity vs. wave number), whereas the corresponding ratio for the spectrum of hecogenin acetate²¹ is roughly 2.5. The sum of the areas of the 900 and 920 cm.⁻¹ bands is roughly the same in the two compounds. When IIa was dissolved in carbon disulfide, carbon tetrachloride or benzene, the appearance of these two bands was similar to Fig. 1. When chloroform, bromoform or pyridine were used as solvents, or when the solid sample was milled in mineral oil, the 900 cm.⁻¹ band was a little stronger than the 920 cm.⁻¹ band, and the second component of the 900 cm.⁻¹ band was not as well resolved. The effect of solvent environment on other lactones of this series was similar. The ester band at 1728 cm.⁻¹ in Fig. 1 includes both lactone and 3-acetate carbonyls. On the other hand the band at 1243 cm.⁻¹ has only an intensity comparable to that of the 3-acetate group of simple sapogenin monoacetates.²¹

Hecolactone 3-Acetate by Hydrogen Peroxide-Acetic Acid Oxidation.—Five grams of hecogenin acetate in 75 ml. of alcohol-free chloroform was dissolved in 30 ml. of glacial acetic acid, 3 ml. of 30% hydrogen peroxide (aqueous) and 2 ml. of 10% sulfuric acid in acetic acid (chilling was not necessary). After 12 days the product was largely diluted with water, was boiled to expel chloroform, and the aqueous layer was decanted from the resulting crystalline crust. The crust was triturated with methanol to give 4.5 g. of hecolactone 3-acetate, m.p. 287–289°, m.p. on recrystallization from acetone 292°. Use of preformed commercial peracetic acid gave a low yield of crude product.

Hecolic Acid (IIIa) (3 β ,13 ξ -Dihydroxy-12,13-seco-5 α ,22a-spirostan-12-oic Acid).—Saponification of hecolactone 3-acetate by refluxing in aqueous methanolic potassium hydroxide for 1.5 hours gave the potassium salt of hecolic acid. The solution was diluted with water, washed with benzene and acidified to precipitate hecolic acid (IIIa) which was crystallized from chloroform to give large needles with a double m.p. 187.5, 253–255°, $[\alpha]^{25}_D -66.3^\circ$ (dioxane).

Anal. Calcd. for C₂₇H₄₄O₆: C, 69.79; H, 9.55. Found: C, 69.76; H, 9.63.

Attempts to acetylate this product gave ring reclosure to hecolactone 3-acetate. The infrared spectrum of the acid

determined on a Kofler block and are therefore corrected. Infrared spectra were obtained with a Perkin-Elmer model 21 spectrophotometer, using a sodium chloride prism.

(22) G. Braun in "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 431.

(solid film on NaCl plate) included strong broad carboxyl band, 2300–3700 cm^{-1} , with peak at 3400 cm^{-1} ; strong, carboxyl carbonyl band at 1700 cm^{-1} with shoulder at 1720 cm^{-1} ; F-ring bands at 866 (m), 901 (s), 922 (m), 983 (s) cm^{-1} , with the usual intensities corresponding to the "iso" configuration.

Hecololactone (IIc) (3 β ,13 ξ -Dihydroxy-12,13-seco-5 α -22a-spirostan-12-oic Acid 12,13-Lactone).—A sample of hecolic acid was heated for 2 hours at 200° (0.15 mm.), to give a neutral product, m.p. 256–258°, $[\alpha]_D^{25}$ –52.0°.

Anal. Calcd. for $\text{C}_{27}\text{H}_{46}\text{O}_6$: C, 72.61; H, 9.48. Found: C, 72.49; H, 9.79.

Infrared spectrum (CHCl₃ solution) included: hydroxyl bands at 3650 cm^{-1} (unbonded) and 3450 cm^{-1} (broad, hydrogen bonded) with the 3650 band much stronger than the 3450 band; lactone carbonyl band at 1705 cm^{-1} ; F-ring bands at 868 (m), 898 (m), 906 (m), 923 (m), 984 (s) cm^{-1} . The 898–906 cm^{-1} band combination is a little stronger than the 923 cm^{-1} band, but the relative intensities are much closer to those of Fig. 1 than to those usually found with "iso" F-ring bands.

Methyl Hecolate (IVa).—Hecolic acid (IIIa), 6.8 g., was dissolved in 75 ml. of tetrahydrofuran (freshly filtered through activated aluminum to remove peroxides and water). The solution was cooled to 0°, a 1.5 M excess of diazomethane in 150 ml. of ether was added and allowed to react for 3 hours during which time the temperature was allowed to rise slowly. A little dilute acetic acid was added to decompose the excess reagent, the solution was diluted with water, extracted with ether, washed with water and the solvents were evaporated on the steam-bath. The glassy residue was crystallized by solution in a minimum of methanol, dilution with cyclohexane and removal of the methanol by distillation of the azeotrope. The product, 6.4 g., melted at 164–165°. Occasionally a metastable form melted about 79°, resolidified on further heating, and remelted at 164–165°, $[\alpha]_D^{25}$ –63.6°.

Anal. Calcd. for $\text{C}_{28}\text{H}_{48}\text{O}_6$: C, 70.26; H, 9.68. Found: C, 70.26; H, 10.14.

Infrared spectrum (CHCl₃ solution) included: hydroxyl bands at 3620 cm^{-1} (unbonded) and 3460 cm^{-1} (broad, hydrogen bonded) of strength corresponding approximately to two hydroxyls per molecule; ester band at 1725 cm^{-1} ; F-ring bands at 866 (m), 900 (s), 922 (m), 982 (s) cm^{-1} corresponding to the usual "iso" F-ring intensities.

Only one of the two hydroxyl groups was acetyltable. The monohydroxy monoacetate, IVb, was obtained as long silky needles, m.p. 99–101°, from pentane, only after slow crystallization, $[\alpha]_D^{25}$ –62.7°.

Anal. Calcd. for $\text{C}_{30}\text{H}_{48}\text{O}_7$: C, 69.20; H, 9.29. Found: C, 69.02; H, 9.33.

Infrared spectrum (CS₂ solution) included: hydroxyl bands at 3600 cm^{-1} (unbonded) and 3510 cm^{-1} (broad, with shoulder at 3450 cm^{-1} , hydrogen bonded) of strength corresponding approximately to one hydroxyl per molecule; F-ring bands at 864 (m), 901 (s), 922 (m), 983 (s) cm^{-1} corresponding to the usual "iso" F-ring intensities; ester band at 1731 cm^{-1} of strength corresponding to two ester groups per molecule; acetate band at 1245 cm^{-1} of strength corresponding to one acetate group per molecule. No band of comparable strength occurs in the 1250 cm^{-1} region of the spectra of IIb, IIc, IIIa, IIIb, IVa, Va, VIa or VII showing that this band must be due to the acetate group. On the other hand, sapogenins known to have two acetate groups have much stronger bands in this region.²¹

Reduction of Methyl Hecolate to Hecolyl Alcohol (Va) (12,13-Seco-5 α -22a-spirostan-3 β ,12,13 ξ -triol).—Methyl hecolate, 2.49 g., in 50 ml. of tetrahydrofuran was slowly added to a slurry of 1 g. of LiAlH₄ in 50 ml. of tetrahydrofuran, and the stirred mixture was refluxed for an additional two hours. The excess reagent was decomposed with water and dissolved in dilute hydrochloric acid, and the product was extracted with ether. The ether layer was washed with water, with 3% sodium hydroxide, and with water. If the sodium hydroxide wash was omitted, traces of hydrochloric acid caused olefin formation during the evaporation of the ether-tetrahydrofuran mixture. The solvents were evaporated to give a colorless glass which formed 2.4 g. of a white solid foam on drying at 80° *in vacuo*. The foam was dissolved in chloroform, and crystalline rhombs precipitated at once. The analytical sample melted at 141° with effervescence, $[\alpha]_D^{25}$ –71.4°.

Anal. Calcd. for $\text{C}_{27}\text{H}_{46}\text{O}_5$: C, 71.96; H, 10.29. Found: C, 72.35; H, 10.09.

Infrared spectrum (CHCl₃ solution) included: hydroxyl bands at 3610 and 3410 cm^{-1} as shown in Fig. 2, dotted curve (see text for discussion); no bands in the 1700 cm^{-1} region. F-ring bands at 868 (m), 902 (s), 923 (m), 983 (s) cm^{-1} corresponding to the usual "iso" F-ring intensities. Dehydration did not occur when the triol was acetylated since hydroxyl bands were observed in the spectrum of the diacetate Vb at 3600 cm^{-1} (unbonded) and 3510 cm^{-1} (bonded). It is interesting to note that the unbonded band of Vb was unusually weak, showing that hydrogen bonding to the acetoxy group is favored in this molecule. The spectrum showed acetate bands at 1720 and 1258 cm^{-1} and F-ring bands at 865 (m), 899 (s), 919 (m), and 981 (s) cm^{-1} .

3-Dehydrohecololactone (VII) (13 ξ -Hydroxy-12,13-seco-5 α ,22a-spirostan-3-one-12-oic Acid 12,13-Lactone).—The triol Va, 2.5 g., was dissolved in 25 ml. of pyridine and was treated with 2.5 g. of CrO₃ dissolved in 40 ml. of cold pyridine and let stand overnight at room temperature. The dark solution was diluted with water and ether, was filtered free of reddish solids, was separated and the ether layer washed well with water, dilute hydrochloric acid, dilute sodium hydroxide and with water. Evaporation of the ether left a colorless, semi-crystalline residue which on trituration with hexane gave 2.0 g. (86% yield) of crystalline product. The analytical sample, hexagonal scales, was crystallized from acetone and from ether, m.p. 250–252°, $[\alpha]_D^{25}$ –51.6°.

Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_5$: C, 72.94; H, 9.07. Found: C, 73.13; H, 9.19.

The infrared spectrum (CS₂ solution) included: a single carbonyl band at 1720 cm^{-1} of strength corresponding to two carbonyl groups per molecule (lactone and ketone); F-ring bands at 865 (m), 896 (m), 905 (m), 921 (m), 982 (s) cm^{-1} with intensity pattern resembling that of Fig. 1.

Dehydration of Hecolyl Alcohol (Va) to Anhydrohecolyl Alcohol (VIa) (12,13-Seco-5 α ,22a-spirostan-3 β ,12-diol).—The triol Va was heated a few degrees above its melting point under a pressure of about 20 mm. and was held at that temperature until effervescence had subsided. The product, a hard colorless glass, was crystallized from acetone to form blades, m.p. 174–176°, $[\alpha]_D^{25}$ –46.1°.

Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_4$: C, 74.95; H, 10.25. Found: C, 74.63; H, 10.33.

Infrared spectrum (CHCl₃ solution) included: hydroxyl bands at 3600 cm^{-1} and 3450 cm^{-1} as shown in Fig. 2, solid line (see text for discussion). The strength of the bonded hydroxyl band is roughly the same as we have observed in natural sapogenins containing only one hydroxyl group at C3. F-ring bands occurred at 867 (m), 903 (s), 921 (m), 983 (s) cm^{-1} . No new strong bands were found in the 900 cm^{-1} region where $\text{R}_1\text{R}_2\text{C}=\text{CH}_2$ should absorb strongly. This suggests that the elimination of the elements of water did not occur between C₁₃ and C₁₈, but that the double bond lies in the D-ring.

Acetylation gave a diacetate, VIb, not crystallized, $[\alpha]_D^{25}$ –35.9° showing no hydroxyl bands in the infrared spectrum and reconvertible on saponification to crystalline VIa. VIb had acetate bands at 1732 and 1243 cm^{-1} of strength corresponding to two acetate groups per molecule, and F-ring bands at 863 (w), 901 (s), 922 (m), 982 (s) cm^{-1} .

Anal. Calcd. for $\text{C}_{31}\text{H}_{48}\text{O}_6$: C, 72.06; H, 9.36. Found: C, 71.84; H, 9.44.

3-Desoxyhecololactone (IIb) (13 ξ -Hydroxy-12,13-seco-5 α ,22a-spirostan-12-oic Acid 12,13-Lactone).—3-Desoxyhecologenin (Ib) (5 α ,22a-spirostan-12-one), 3.8 g., m.p. 190–195°, in 70 ml. of chloroform, was oxidized at room temperature with 70 ml. of a solution of perbenzoic acid in chloroform (50 mg./ml.) in the presence of 4 ml. of 10% sulfuric acid in acetic acid. After 64 hours the chloroform solution was washed with water, dilute sodium hydroxide, and water, and was evaporated on the steam-bath. Crystallization from acetone gave 3 g. of the pure compound, m.p. 240–241°, $[\alpha]_D^{25}$ –5.85°.

Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{O}_4$: C, 75.31; H, 9.83. Found: C, 75.14; H, 9.51.

Infrared spectrum (CS₂ solution) included: lactone band at 1727 cm^{-1} and F-ring bands at 864 (m), 898 (m), 906 (m), 923 (m) and 983 (s) cm^{-1} with intensity pattern resembling that of Fig. 1. Assignment of a band to the lactone function in the 1200–1300 cm^{-1} region is difficult since greater

differences between the spectra of IIa and IIb are found here than between the analogous compounds tigogenin acetate and 3-desoxytigogenin.²³

3-Desoxyhecolic Acid (IIIb) (13 ξ -Hydroxy-12,13-seco-5 α -22a-spirostan-12-oic Acid).—Saponification of IIb gave 3-desoxyhecolic acid. The product melted at 178° on a preheated Kofler stage, solidified on further heating, and remelted at 238.0–241.4°, $[\alpha]_D^{25}$ –63.8°.

Anal. Calcd. for C₂₇H₄₄O₆: C, 72.28; H, 9.89. Found: C, 71.84; H, 9.99.

Infrared spectrum (CHCl₃ solution) included: broad carboxyl-hydroxyl absorption in the 3000 cm.⁻¹ region, carboxyl-carbonyl band at 1707 cm.⁻¹, and F-ring bands at 868 (m), 901 (s), 923 (m), 984 (s) cm.⁻¹ with the usual intensity pattern for "iso" F-rings.

Conversion of Methyl 3 α -Carbethoxyoxy-12-ketocholanate to Methyl 3 α -Carbethoxyoxy-13 ξ -hydroxy-12-carboxy-12,13-secocholanate 12,13-Lactone (X).—A sample of methyl 3 α -carbethoxyoxy-12-ketocholanate, m.p. 155–159°, 5 g., was oxidized during a ten-day reaction period at 25° using as oxidant 60 ml. of perbenzoic acid in chloroform solution (50 mg./ml.) containing 2 ml. of 10% sulfuric acid in acetic acid. The product was obtained after the usual washing with water, dilute sodium hydroxide and water, and evap-

oration of solvent as a glassy residue which crystallized on rubbing with pentane. This procedure gave 5.04 g. of a crystalline crude product, m.p. 98–102°. A portion chromatographed on SiO₂·xH₂O was eluted with chloroform to give the analytical sample, m.p. 105–106.5°, $[\alpha]_D^{25}$ +2.95°. No other steroidal materials were formed in the reaction.

Anal. Calcd. for C₂₈H₄₄O₇: C, 68.26; H, 9.00. Found: C, 68.23; H, 8.94.

The lactonic product, without isolation of intermediates, was sequentially saponified, esterified with diazomethane, and reduced with LiAlH₄ to a polyhydroxy compound which on acetylation showed unacetylated hydroxyl infrared bands. The infrared spectra of the intermediate crudes were more difficult to interpret than the corresponding sapogenin analogs because of the overlapping of the absorption bands of the functional groups.

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